PhD THESIS

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PhD Thesis

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EFFECT OF DIETARY STARCH AND NDF CONTENT AND SUPPLEMENTATION OF MANNANOLIGOSACCHARIDE AND INULIN ON THE PERFORMANCE AND SOME PHYSIOLOGICAL PARAMETERS OF CALVES REARED ON MILK REPLACER

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1. Background and objectives of the research

Raising calves and heifers represents a significant proportion of the costs of a dairy farm without making a profit during the long period of rearing. The aim of calf rearing is to minimize losses, ensure the health and resilience of the animals, reduce rearing time and due to these terms increase the efficiency of the rearing.

Achieving these goals is hampered by several factors. The mortality rate of dairy calves (3.4-8.1%) has hardly changed worldwide in the last 10 years, despite significant improvements in technology and farm management. The primary cause of death is gastrointestinal problems and a lack of passive immunity in the first few weeks of life (Wilde, 2008). Following the ban on antibiotics administered with prophylactic feed, there has been a growing demand for alternative feed supplements that support the vitality and performance of calves by ensuring digestive health.

Another basic task of calf rearing is to help the development of the foregut, including rumen digestion, by using appropriate feeding technology. Fiber sources are essential for the healthy development and function of the rumen, but the lower fiber content of the feed improves feed efficiency (Terré et al, 2013). However, it should be noted that higher amounts of rapidly degradable carbohydrates able to reduce rumen pH, which is unfavourable for bacteria living in the rumen. There is no consensus on the composition, nutrient content, level of fiber and starch of starter feeds. The scientific results are contradictory.

In my doctoral work, I focused on that field of calf rearing, which plays a significant role in the growth of animals, their loss-free rearing and the development of their rumen function.

We conducted studies on two issues.

1: How the composition of the concentrate influences the development of rumen function in calves and the growth of animals (experiment 1.). In this context, we aimed to answer the question that:

- how the different carbohydrate source (starch, hemicellulose) of the calf's starter diet affects the growth and certain physiological and microbiological parameters of the calves,
- does a mixture of different compositions concentrate fed during different periods of calf rearing have a growth promoting effect on the whole raising period?

To answer the questions, we examined what changes are induced by a different carbohydrate source:

- in body weight gain and feed intake of calves
- in the ammonia content, concentration of the short chain fatty acid (SFCA) and the composition of microbiota of the rumen fluid
- certain blood clinical-chemical parameters (albumin, urea, glucose, triglycerides).

2. A further aim of my doctoral dissertation was to study the inulin, which is less studied in calves, and the prebiotic MOS, which has a different mechanism of action than usual prebiotic source (experiments 2 and 3). We hypothesized that one reason for the controversy in the scientific literature regarding the efficacy of the prebiotic is the low dose. Therefore, in both studies, supplementation (18.7 and 28 g/day) greater than that described there in (2-12 g/day) was used.

I wanted to study how MOS and inulin at different concentrations and at different ages (during the whole calf raising [60 days] and the first 14 days of life) affect the:

- body weight gain and feed intake of calves
- microbial composition of faeces
- certain blood clinical-chemical parameters (albumin, cholesterol, urea, glucose, total protein, triglycerides, bilirubin, creatinine).

Changes in some immunological parameters were also planned to be monitored during the first two weeks of life when larger amounts of MOS and inulin supplementation was given with colostrum and milk replacer (Experiment 3). The focus of the study was on the early period of calf rearing (0–14 days) during which the greatest support is needed to establish adequate immune status and intestinal microbiome. In addition to measuring blood IgG levels, the aim of this study is to determine whether the ovalbumin-induced immune response in calves is altered by MOS or inulin supplementation.

When designing the experiment, we sought to obtain data on the changes caused by the treatments according to uniform criteria. Therefore, in each experiment, we measured the effect of the treatments on the weight gain as well as the natural parameters that indicate the effectiveness of calf rearing. These data were also used to monitor and confirm age-related physiological changes due to repeated sampling.

2. Material and methods

The experiments were carried out at the dairy farm of the Bos-Frucht Agricultural Cooperative in Kazsok, Hungary. The selection and placement of experimental animals was the same in all three experiments.

In each experiment, we measured the natural parameters which indicate the efficiency of calf rearing:

- feed intake
- body weight
- body weight gain

As well as the effect of each treatment on the metabolism:

- clinical-chemical parameters of blood
- bacterial composition of faeces or rumen fluid

The aim of the Experiment 3 was to map the effect of the prebiotics supplementation was given by colostrum or with milk replacer in early age (0-14 days) on the immune system. To do this, the following tests were performed:

- IgG supply
- Changes in IgG levels due to a specific antigen - provocation of an immune response with ovalbumin
- Lymphocyte stimulation test (LST)

The data were also used to monitor and confirm age-related physiological changes due to repeated sampling.

The structure of the three experiments and the schedule of the sampling are described in Figures 1, 2 and 3, and then I present the feeding aspects.

2.1. Effect of feeding calf starter with different starch and NDF level on calf performance and physiological parameters (Experiment 1)

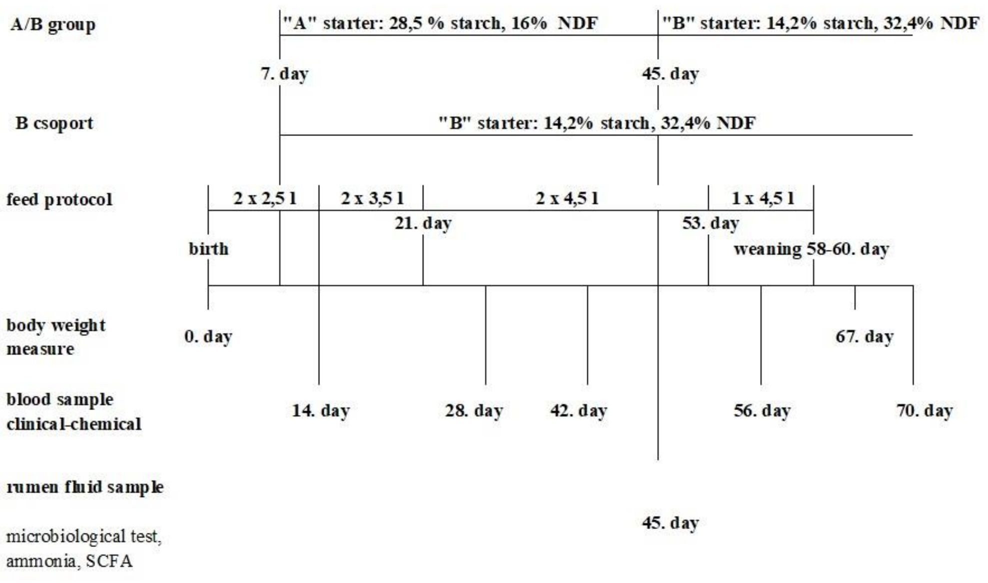

Figure 1: Structure of Experiment 1 and schedule of sampling

Sixty Holstein Friesian (HF) heifer calves were randomly divided into two groups at birth (n = 30 / group), the average birth weight of the calves was 40.1 ± 3.4 kg.

The feeding protocol was administered based on the technology used at the dairy farm. Milk replacer, Sprayfo Yellow (Sloten Group BV, The Netherlands) diluted in 12.5% were given twice a day (7.00 and 19.00) according to the amounts shown in Table 2. to the calves from bucket from 12 hours to 21 days after birth and then a mixture of whole milk and milk replacer from day 21 to weaning (75% milk replacer + 25% pasteurized whole milk).

1. **Table: Feeding protocol administered in Experiment 1**

Age (day)	Quantity (l)	g milk replacer/day
1-14	2 * 2,5	625
15-21	2 * 3,5	875
22-53	2 * 4,5	750
54-60	1 * 4,5	375

Two starter feeds ("A", "B") with different starch and NDF contents were fed. In the starter feed "A" higher starch content was used while the amount of each fiber fraction (NDF, ADF) was smaller (28.5% starch and 16% NDF). Conversely, starter "B" had a lower starch content and a higher NDF content (14.2% starch and 32.4% NDF). In group A/B, the calves were fed in two phases. In the first phase (days 7-45), calf feed "A" was fed, followed by calf feed "B" in the second phase (days 46-70). Group B received single-phase feeding (days 7-70) with starter "B".

2.2 Effect of MOS and inulin supplementation with milk replacer during rearing on calf performance and physiological parameters (Experiment 2)

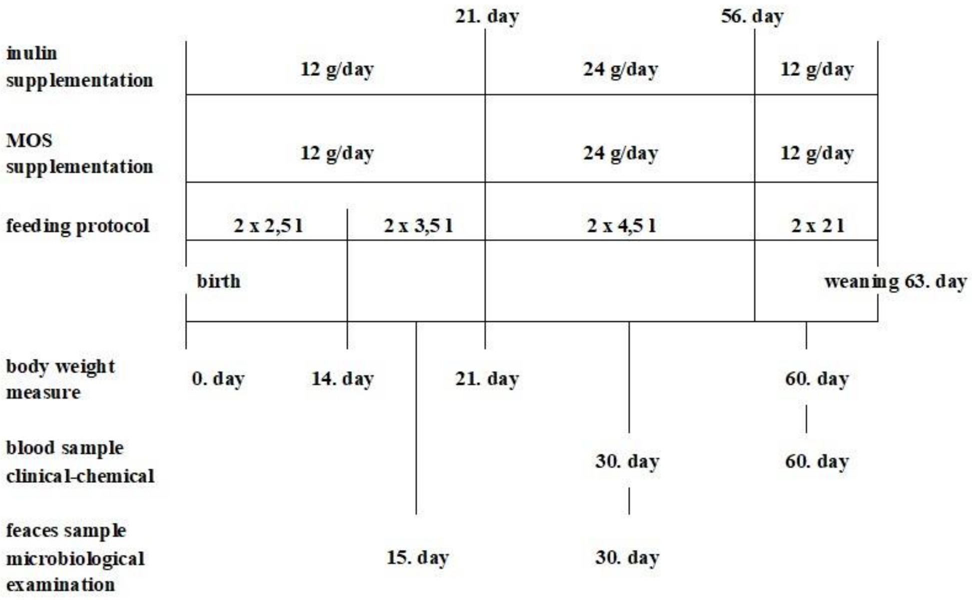

Figure 2. Structure of Experiment 2 and schedule of sampling

For the study, 45 Holstein Friesian heifers (birth weight: 39.2 ± 3.6 kg) were included in the experiment. Sprayfow Yellow (Sloten Group B.V., The Netherlands) milk replacer was used to raise the calves at a dilution ratio of 145 g/l. The 45 calves (3x15 animals) in the study were randomly assigned to one of the three experimental groups the day after their birth (1. Control without prebiotic supplementation 2. MOS supplementation 3. Inulin supplementation). The mannanoligosaccharide and inulin were mixed with the milk replacer. According to the age of the calves and the farm practice, the amount of milk replacer and prebiotics was varied as follows (Table 2):

Table 2: Feeding protocol used in Experiment 2 and amount of prebiotic supplementation

Age (day)	Quantity (l)	g milk replacer/day	MOS or inulin supplementation (g/day)
1-14	2 * 2,5	725	12
15-21	2 * 3,5	1015	12
22-56	2 * 4,5	1305	24
5-63	2 * 2	580	12

The increase in prebiotic dose between 22 and 56 days of age was justified by the results of previous experiments (Diaz et al., 2001; Jasper and Weary, 2002) related to milk replacer consumption and diarrhea in calves. According to these authors, feeding large amounts of milk or milk replacer (> 9 l/day) may increase the incidence of diarrhea. Thus, in order to reduce the negative effects of milk replacer, the original dose of the prebiotic (12 g/day/calf) was doubled (24 g/day/calf). Subsequently, as the amount of milk replacer decreased during the weaning period, they were again given 12 g/day of prebiotic. Based on the doses discussed above, calves received an average of 18.7 g of MOS and inulin supplementation throughout the feeding period. Calves were weaned between 54 and 63 days of age.

2.3. Effect of MOS and inulin supplementation on calf performance and physiological parameters in calves with colostrum and milk replacer (Experiment 3)

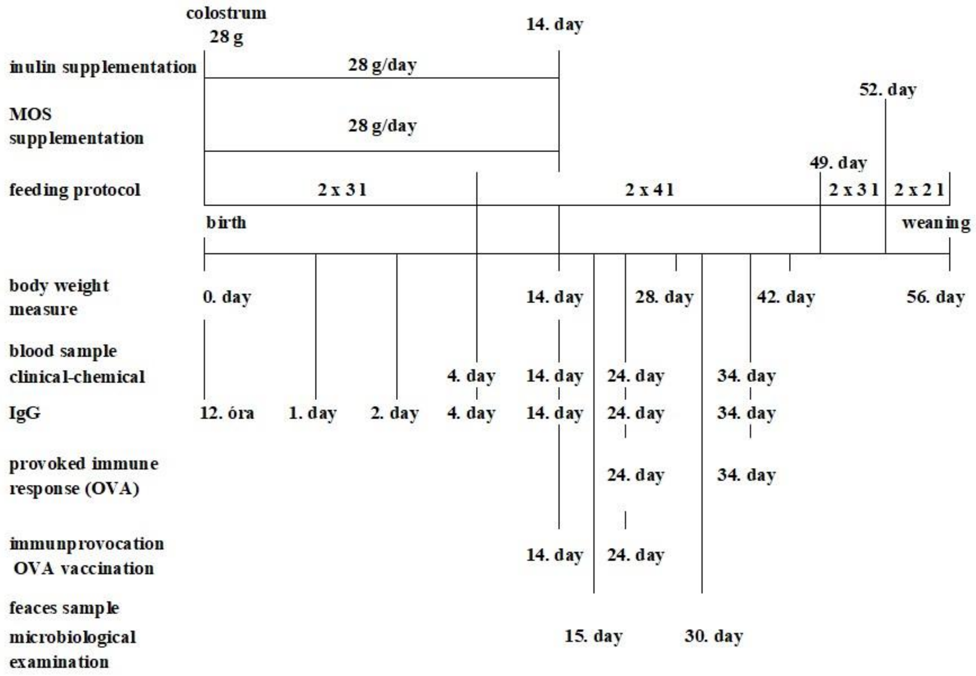

Figure 3. Structure of Experiment 3 and schedule of sampling

For the study, 30 HF heifer calves (birth weight: 38.5 ± 3.9 kg) were divided into 3 groups with 10-10 calves per group (control without prebiotic supplementation and groups receiving MOS supplementation and inulin supplementation, respectively). Prebiotics were administered at 28 g/day with colostrum and milk replacer for 0-14 days. After a single dose of colostrum milk, the calves received 140 g/l of mixed milk replacer from a bucket twice a day at 12-hour intervals (Nukamel Performer, Nukamel, Weert, Belgium). The feeding technology used for calves is described in Table 3.

Table 3: Feeding protocol used in Experiment 3

Age (day)	liter/day/calf	g milk replacer/day
1-4	2*3	840
5-48	2*4	1120
49-52	2*3	840
53-56	2*2	560

2.4. Statistical analysis

In the experiments, heifers were divided into equal groups according to the treatments in a random arrangement. All other conditions (e.g., housing, feeding system) were the same for all animals during each study, except for prebiotic supplements (Experiments 2 and 3) and feeding a different carbohydrate starter diet (Experiment 1). RcmdrPlugin.aRnova from R Commander (Version 3.4.1, 1991) was used to perform statistical analyzes. For the given parameters, the arithmetic mean, standard deviation (SD), standard error of the means (SEM) were calculated for each group using the descriptive statistics of the program. The normality of the data distribution was checked by the Saphiro-Wilk method. According to the number of treatments, in Experiment 1, the two groups were evaluated by two-sample t-test or Kruskal-Wallis test, depending on the distribution of the data, while in Experiments 2 and 3, the three groups were evaluated by analysis of variance (Repeated Meauseres of ANOVA). The correlation test of the program was used to examine the correlations between volatile fatty acid and microbial composition. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of feeding calf starter with different starch and NDF level on performance and physiological parameters (Experiment 1)

No difference was found in the milk replacer consumption of calves during the study. The type of carbohydrate source in the feed had a significant ($P < 0.05$) effect on the feed intake of calves between 2 and 5 weeks of age. During this period, calves consumed higher amount of starter diet with higher starch (28.5%) and lower NDF (16%) than those with lower starch (14.2%) and higher NDF (32.4%). In the second half of the study (45-67 days), there was no significant difference in feed intake between the groups.

The different carbohydrate starters fed during the first 45 days did not affect the body weight of the calves (age 67 days) and their weight gain during the rearing period (539 ± 110 and 537 ± 112 g/day). The increased feed intake observed in the first phase of the study had no effect on body weight.

The different NDF and starch contents of the starter diet did not affect the number of bacteria tested in the rumen, had no affect the ratio of acetic acid to propionic acid in the rumen fluid or the concentration of total short chain fatty acids and ammonia.

Feeding a starter diet with different level of carbohydrate sources did not affect blood glucose, triglyceride, and urea concentrations. An interaction between treatment and age was observed for albumin ($P < 0.01$). The albumin levels of calves consuming a higher starch-containing starter diet were higher on day 28 and lower on day 56 than those of their counterparts consuming a higher NDF-containing starter. In the “A / B” group, the albumin concentration peaked on day 28 and in the “B” group on day 56. We may have found a significant difference between the samples taken at different time points, but the changes in the values are not related to a natural indicator (appetite, body weight). Summarizing the results of albumin analysis on all sampling days ($n = 5$), no significant differences were found between the groups, the mean values were 31.51 g/l and 31.33 g/l, respectively.

3.2. Effect of MOS and inulin supplementation with milk replacer during rearing on calf performance and physiological parameters (Experiment 2)

Supplementation of the milk replacer with a prebiotic did not affect the solid feed intake of the calves at any time of life.

Feeding of prebiotics did not alter the live weight and daily body weight gain of the calves at any of the administered doses. Thus, neither the 12 g prebiotic administered at the beginning of the study (0-21) nor the 24 g/day prebiotic from day 21 affected calf growth.

Prebiotics did not affect the number of *E. coli* and *coliforms* or *Lactobacilli* in the faeces. *Clostridia* were present in higher amounts in the faeces of calves consuming inulin than in calves in the control and MOS groups. Inulin increased the number of Bacteroidetes compared to MOS, but the difference is not significant compared to the control group. The anaerobic bacteria count of calves consuming inulin-supplemented milk replacer was higher than that of those receiving MOS supplementation, but none of the groups differed from the control group. The aerobic germ counts also increased in the faeces when feeding inulin compared to the control group. MOS had no effect on this parameter. Regarding the age of the calves, it can be stated that the number of faeces *Clostridium* and total aerobic bacteria counts were significantly lower on day 30 compared to day 15.

Inulin supplementation resulted in lower total protein concentrations in the blood serum of calves than in the other two groups. Concentration of albumin, cholesterol, glucose, triglyceride, and urea was unchanged when prebiotics were fed. No treatment-time interaction was observed in any of the values tested.

3.3. Effect of MOS and inulin supplementation on calf performance and physiological parameters in calves with colostrum and milk replacer (Experiment 3)

No change in feed intake was observed with 28 g/day MOS or inulin supplementation between 0 and 14 days.

No significant difference in live weight was found between groups at any of the measurement time points. However, in the average weight gain in the first two weeks of life and between day 28-42, there was a significant difference and the whole period of calf rearing (days 0-56). In the period between 0 and 14 days, the weight gain of calves was significantly lower when feeding inulin ($P < 0.05$) compared to the control group. These calves were not able to compensate for the lack of growth in further part of the calf raising. For the entire study period (0-56 days), the daily weight gain of calves in the inulin group was 647 g, compared to 776 g in the control group ($P < 0.05$). Due to the weaker growth capacity, the average body weight of inulin-treated calves was the lowest in the groups, and this difference may not have been significantly different on any of the days of life studied. MOS supplementation did not affect the growth of the calves, the values did not show a significant difference compared to the control group.

MOS and inulin supplementation up to 14 days of age did not affect faecal *Clostridium*, *Bacteroidetes*, *Lactobacillus* and total anaerobic and total aerobic bacteria counts. The number of faecal *E. coli* and *coliforms* in MOS-supplemented calves was significantly higher than in the control and inulin-treated groups ($P < 0.05$).

None of the treatments resulted in significant differences in blood albumin, cholesterol, total protein, triglyceride, and urea concentrations. The glucose levels of the calves in the MOS-supplemented group were higher than those of the inulin-treated group, but none differed from the values of the animals in the control group. Creatinine concentrations were significantly higher in both prebiotic-supplemented groups compared to the control group.

IgG level in inulin-treated calves was found to be lower than in control or MOS-treated calves. IL-6 cytokine production in inulin-treated calves lags behind cytokine production in control and MOS-treated animals ($P < 0.05$). OVA-specific IgG, IL-1β, IL-2, and IFN-γ cytokines showed no treatment-dependent change. Although no significant interaction was observed for immunological parameters between treatment and time, inulin significantly increased IL-1β production by day 24 after the first OVA vaccine compared to MOS.

4. Conclusions

According to our results, the increase in the solid feed intake of calves is more affected by the starch and NDF content of the feed mixture than the MOS or the inulin supplementation with liquid feed. Prebiotics (MOS, inulin) administered with milk replacer in different amounts (18.7 or 28 g/day) and for a different period of time (60 days or 14 days) do not affect feed intake, weight gain or feed sales. The beneficial growth promoting effect of prebiotics on weight gain, known from the literature, has not been demonstrated at higher doses than those described therein. Based on our experimental results and literature data, it can be assumed that the growth promoting effect of prebiotics is influenced by factors other than the daily dose. I believe that if the production results of the control group (weight gain, feed intake) are as expected according to the applied feeding technology, the prebiotic will not be able to further increase performance. However, it should be considered that by stabilizing the intestinal flora, MOS and inulin contribute to the reduction and prevention of damage caused by environmental factors that adversely affect the development of calves. Due to the latter, the administration of prebiotics for preventive purposes is justified.

Examining the faecal microbiota composition of our experiments with prebiotics supplementation, we found that, using prebiotics during the entire rearing period (60 days), with advancing age only the number of *Clostridium* and aerobic bacteria changed. However, when prebiotic supplementation was applied only until day 14, the values of all other microbiological parameters except for anaerobic bacteria count changed with age. These results suggest that administration of prebiotics over the entire raising period may reduce changes in the composition of the intestinal microbiota with age.
The effect of MOS on faecal microbial composition and number may be due to the adsorption properties of MOS. When examining feces, it is not possible to distinguish whether the number of pathogen bacteria adhering to the colonic mucosa and colonizing there actually increased, or the excretion of pathogens increased because they were more bound by MOS. Greater colonization of pathogen bacteria is a more serious problem in terms of antigen loading in animals. A comparative analysis of the relevant test methods can help to understand this process more accurately.

5. New scientific results

1. In the first five weeks of a calf's life, solid feed intake is affected by the starch and NDF content of starter feeds. In order to increase feed intake during this period, it is preferable for the starter feed to contain more starch (28.5%) and less NDF (15.9%).

2. During the 60-day rearing period, the 18.7 g/day MOS and inulin supplementation of the milk replacer had no effect on the body weight gain and feed intake of the calves, faecal number of *Lactobacillus*, *E. coli* + *coliform* and *Bacteroidetes* and the clinical chemistry blood parameters tested.

3. Daily 18.7 g of inulin supplementation in milk replacer up to the age of 60 days significantly increased the number of *Clostridium* and aerobic bacteria in

the faeces, respectively, compared to the control.

4. In the first two weeks of the calves' life, the addition of 28 g of inulin per day was significantly reduced the daily weight gain compared to the control, and the 28 g of MOS per day did not affect the body weight of the calves during the whole rearing period (days 1-56). None of the prebiotics had a significant effect on the feed intake of the calves during the entire rearing period.

5. In the first two weeks of the calves' life, the addition of 28 g of inulin per day to the colostrum and milk replacers of the calves had a lower total IgG level in the blood serum. In the immune response provoked by ovalbumin (OVA) on days 14 and 24, neither inulin nor MOS affected the production of OVA-specific IgG and IL-1β, IL-2 or IFNγ compared to the control group. However, in animals in the inulin-treated group the IL-6-expressing gene showed changes due to the supplementation.

6. Suggestions

The following suggestions can be made for the usability of the results:

- In the first 5 weeks of life, it is recommended to use a starter diet with a lower NDF (15.9%) content in addition to a higher starch (28.5%) content to promote higher concentrate intake of calves.
- The use of 28 g of inulin supplementation per day for the first two weeks of life in calves is not recommended due to its depressive effect on weight gain.
- Longer-term use of MOS or inulin over the entire duration of milk feeding (18.7 g / day) may be justified by favourable changes in the composition and number of intestinal microbiota.

7. Scientific publications on the topic of the dissertation; other publications; presentations

Publication in a peer-reviewed foreign language journal

Tóth Sz., Kovács M., Bóta B., Szabó-Fodor J., Bakos G., Fébel H. (2019) Effect of the composition of starter diet fed in the rearing phase on the performance and certain physiological parameters of Holstein calves. Czech Journal of Animal Science 64. 9. 367-376. DOI: https://doi.org/10.17221/34/2019-cjas

Tóth Sz., Kovács M., Bóta B., Szabó-Fodor J., Bakos G., Fébel H. (2020) Effect of mannanoligosaccharide (MOS) and inulin supplemention on the performance and certain physiological parameters of calves reared on milk replacer. Journal of Applied Animal Research 48. 1. 228-234. DOI: https://doi.org/10.1080/09712119.2020.1770096

Publication in a peer-reviewed journal in Hungarian

Tóth Sz., Kovács M., Fébel H. (2018) The effect of mannan oligosaccharide (MOS) on the growth of calves, the composition of the intestinal flora, the immune system, its role in the reduction of enteric diseases. Literature review Állattenyésztés és Takarmányozás 67. 2. 63-77.

Tóth Sz. (2018) Regulation of the use of pre- and probiotics in the European Union and other countries around the world. Acta Agraria Kaposváriensis 22. 2. 46-62 DOI: https://doi.org/10.31914/aak.2278

Tóth Sz., Kovács M., Fébel H. (2020) The role and importance of inulin in the feeding of farmed animals. Acta Agraria Kaposváriensis 24. 1. 55-66. DOI: https://doi.org/10.31914/aak.2372

Proceeding in full in a foreign language

Tóth Sz. (2019) The effect of the quantity and quality of milk replacer intake

on starter feed intake in Holstein calves. Biologiya Tvaryn / Animal Biology 21. 2. 70-72. DOI: https://doi.org/10.15407/animbiol21.02.070

Tóth Sz. (2019) Effect of mannanoligosaccharide (mos) and inulin supplementation on the performance of calves reared on milk replacer. Review on Agricultural and Rural Development 8. 1-2. 81-84.